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Effect of rabeprazole on histamine synthesis in enterochromaffin-like cells of mast cell-deficient (Ws/Ws) rats

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Abstract

The effect of rabeprazole, the latest proton pump inhibitor, on the serum gastrin concentration, histidine decarboxylase activity and histamine content of the oxyntic mucosa in Wistar rats, mast cell-deficient (Ws/Ws) rats, and their normal type, +/+, rats was investigated. In Wistar rats, 2 weeks of treatment with rabeprazole (30 mg/kg/day, s.c.) induced a 1.8-fold increase in serum gastrin concentration and a 3.9-fold increase in histidine decarboxylase activity of the oxyntic mucosa over the control levels, whereas neither 2- nor 4-week treatment affected the histamine content of the oxyntic mucosa. In Ws/Ws and +/+ rats, the serum gastrin concentration, histidine decarboxylase activity and even histamine content of the oxyntic mucosa were increased significantly as compared with control levels after the 4-week treatment with rabeprazole. Immunohistochemistry using a histamine antibody confirmed the increase in the histamine content of the oxyntic mucosa after the 4-week treatment with rabeprazole. The finding that there were no differences in serum gastrin concentration and histidine decarboxylase activity between Ws/Ws and +/+ rats, both with and without the 4-week treatment, indicates that mast cells do not respond to endogenous hypergastrinemia elicited by acid-inhibitory treatment. Moreover, the present study clarified for the first time that enterochromaffin-like (ECL) cells in Ws/Ws rats synthesize and store histamine in response to gastrin.

Keywords: Histamine; Enterochromaffin-like cell; Mast cell; Rabeprazole; Mast cell-deficient, rat

1. Introduction

Gastric histamine in the rat is stored in the so-called enterochromaffin-like (ECL) cells and mast cells. The former are located in the basal half of the oxyntic mucosa and the latter in the superficial layer of the oxyntic mucosa and in the submucosa (Håkanson et al., 1986a). The ECL cells form an interface between the peripheral and central regulation of acid secretion. Their main role is the secretion of histamine, which is predominantly activated by gastrin (Håkanson et al., 1977; Sandvik et al., 1987; Prinz et al., 1993). Moreover, ECL cells respond to gastrin in a time-dependent manner. The immediate response of ECL cells to gastrin challenge is the release of histamine within minutes (acute phase). Later, there is activation of the histamine-forming enzyme, histidine decarboxylase, within hours (intermediate phase). Still later, if the gastrin stimulus persists, ECL cells respond with hypertrophy after several days, hyperplasia after weeks or months, and finally even dysplasia/neoplasia after 1–2 years (chronic phase) (Larsson et al., 1986, 1988; Håkanson et al., 1986b; Chen et al., 1994). The term gastrin–ECL cell axis has been introduced to emphasize the close relationship between gastrin and ECL cells (Chen et al., 1994).

The precise role of histamine derived from mast cells in the stomach is not clear and it has not been defined whether there is a mutual relationship between ECL cells and mast cells, although both cells release the same transmitter, histamine. Thus far, when estimating the effect of any drug or experimental load on ECL cells, especially when measuring the histamine content of the oxyntic gland of the stomach, it has been difficult to obtain precise data because scraped samples from the oxyntic mucosa always include all mast cells in the superficial layer of the oxyntic

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mucosa and to some extent the mast cells in the submucosa. In the present study, we were able to overcome this problem by means of mast cell-deficient mutant rats (Ws/Ws rats). The Ws/Ws rats, which have a small deletion at the tyrosine kinase domain of the c-kit gene, are deficient in both mucosal-type mast cells and connective tissue-type mast cells (Niwa et al., 1991; Onoue et al., 1993).

Rabeprazole sodium is the latest member of a class of substituted benzimidazole molecules known as proton pump inhibitors. Its potent antisecretory effect and subsequently induced hypergastrinemia have been demonstrated (Fujisaki et al., 1991; Kawai et al., 1992; Tomiyama et al., 1994). Rabeprazole was administered to Wistar, Ws/Ws, and their wild type, +/+, rats in order to evaluate its effect on histidine decarboxylase activity, histamine content and the density of ECL cells in the oxyntic gland area of the stomach.

This study addresses the question of whether mast cells in the rat stomach are associated with the gastrin–ECL cell axis.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 240–250 g, were purchased from CLEA Japan (Osaka, Japan) and kept in our laboratory. Male and female Ws/+ rats, both of the Donryu strain, were crossed to obtain male Ws/Ws rats, which are deficient in mast cells, and their wild type, +/+ rats, using the procedure described by Niwa et al. (1991). They were cared for in accordance with the principles and guidelines of the Animal Care Committee of Ehime University, School of Medicine. Both the Ws/Ws rats and +/+ rats weighed 150–200 g at the start of administration of the drug. All of the rats were housed at a constant temperature (24 ± 2°C), with a humidity of 55 ± 10% and an automatically controlled 12:12-h light/dark cycle (lights on at 7:00 am). The rats had free access to food and water. They were fasted for 24 h before being killed, but were permitted intake of water ad libitum. All experiments were carried out in the daytime.

2.2. Drug treatment and preparation of the blood and stomach samples

Sodium rabeprazole was kindly provided by Eisai Pharmaceuticals (Tokyo, Japan). Sodium rabeprazole was dissolved in saline solution and administered to Wistar rats, Ws/Ws rats and +/+ rats subcutaneously (30 mg/kg) once daily for 2 weeks or 4 weeks (six rats for each treatment period) (Tomiyama et al., 1994). Control rats received the same volume of saline solution.

The fasted rats were anesthetized with 1.2 g/kg of urethane intraperitoneally. Blood was drawn from the abdominal aorta. Serum was stored at −20°C until further analysis. The stomach was removed, opened along the major curvature and rinsed in saline. Three small specimens (4 mm diameter) from the oxyntic gland area of each stomach were collected for histamine immunohistochemistry. For determination of the histamine content and histidine decarboxylase activity, the mucosa from the remaining part of the oxyntic gland area of the stomach was scraped off and stored at −80°C.

2.3. Determination of serum gastrin concentration

The serum gastrin concentration was measured by a radioimmunoassay method using a gastrin RIA Kit (Dainabot, Tokyo, Japan). The level is expressed as picogram equivalents of human gastrin-17 per milliliter of serum.

2.4. Determination of histamine content and histidine decarboxylase activity of the oxyntic mucosa

The concentration of histamine in tissue homogenates was measured by a high performance liquid chromatography (HPLC)–fluorometry technique (Yamatodani et al., 1985; Guo et al., 1997). Each tissue preparation of oxyntic mucosa was weighed and then promptly homogenized in 10 vol. of ice-cold histidine decarboxylase solution (0.1 M potassium phosphate buffer, pH 6.8, which contained 0.01 mM pyridoxal 5′-phosphate, 0.2 mM dithiothreitol, 1% polyethylene glycol, and 100 μg/ml of phenylmethylsulfonyl fluoride) in a Polytron homogenizer (Kinematica, Luzern, Switzerland). One hundred microliters of the homogenate was mixed with 900 μl of 0.46 M perchloric acid. After centrifugation at 10,000 × *g* for 15 min at 4°C, 20 μl of the supernatant was diluted 10 times with 5 mM Na_2EDTA. Fifty microliters of this solution was used for HPLC–fluorometry. Histamine content is expressed as nanomoles per gram wet weight. The remaining homogenate was used to determine histidine decarboxylase activity as mentioned previously (Watanabe et al., 1980; Sugimoto et al., 1995). All of the remaining homogenate was centrifuged at 10,000 × *g* for 15 min at 4°C, and the supernatant was dialyzed three times against 100 vol. of histidine decarboxylase solution at 4°C. The enzyme solution was incubated with 0.25 mM L-histidine for 1 h at 37°C in 0.5 ml of histidine decarboxylase solution. After brief centrifugation of the reaction mixture, the histamine in the supernatant was measured by HPLC–fluorometry. Histidine decarboxylase activity is expressed as the formation of histamine picomoles per minute per milligram protein. The protein content of each enzyme solution was determined by using a Bio-Rad protein assay kit (Bio-Rad, Richmond, USA); bovine serum albumin was used as the standard.

2.5. Histamine immunohistochemistry

Tissue specimens from the oxyntic gland area were fixed by immersion in 4% 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (ICN Biomedicals, Aurora, Ohio, USA) in 0.1 M sodium phosphate buffer (pH 7.4) for 8–12 h at 4°C, rinsed with 10% sucrose in 0.1 M sodium phosphate buffer overnight, and then frozen in OCT embedding medium (M-1 embedding matrix, Lipshaw, Pittsburgh, PA, USA) at −80°C (Nissinen and Panula, 1993). Frozen sections(7 μm) were cut in a cryostat and washed three times in 0.05 M Tris–HCl containing 0.15 M NaCl (Tris-buffered saline, pH 7.6). The sections were incubated overnight at 4°C with rabbit histamine antiserum (Code B 80-1, Eurodiagnostics, Malmö, Sweden) (Lindström et al., 1997) diluted 1:300 in Tris-buffered saline containing 1% bovine serum albumin followed by washing for 20 min. Immunoreaction was visualized by incubation with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin G (Sigma, St Louis, MO, USA) diluted 1:40 in Tris-buffered saline for 2 h at room temperature. The sections were washed and mounted in aqueous mounting medium with anti-fading agents (Biomeda, Foster City, CA, USA) and examined with a fluorescence microscope. Each of the sections was photographed at the magnification used (×100) on 35-mm color reversal film. Captured image data sets were digitized using a film scanner (LS-1000, Olympus, Tokyo, Japan), transferred to a personal computer, and then analyzed with a computer image analysis program (NIH Image, version 1.61). The areas that showed histamine immunofluorescence were segmented from background objects according to mean grayscale intensity and pixel area, colored red using a density slice function, and then measured. The area colored red was determined as a percentage of the oxyntic mucosal area traced manually. Oxyntic mucosal thickness was also measured.

2.6. Statistical analysis

All data are expressed as means ± SEM. ($n = 6$ in each experimental group of rats). One-way analysis of variance, followed by the Fisher test, was used to determine whether the difference between two groups was statistically significant. A probability value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of rabeprazole on serum gastrin concentration, oxyntic mucosal histidine decarboxylase activity and histamine content in Wistar rats

Fig. 1a shows the effect of rabeprazole on the serum gastrin concentration in Wistar rats. Two weeks of treatment with rabeprazole induced a 1.8-fold increase in serum gastrin concentration over control levels (treated vs. control: 706.38 ± 101.60 pg/ml vs. 383.56 ± 44.29). After 4 weeks of treatment, gastrin levels were 4.2-fold higher than control levels (872.81 ± 173.28 vs. 209.58 ± 24.12). Compared with the 2-week treatment study, treatment for 4 weeks caused no further significant elevation in gastrin levels, in either treated or control rats.

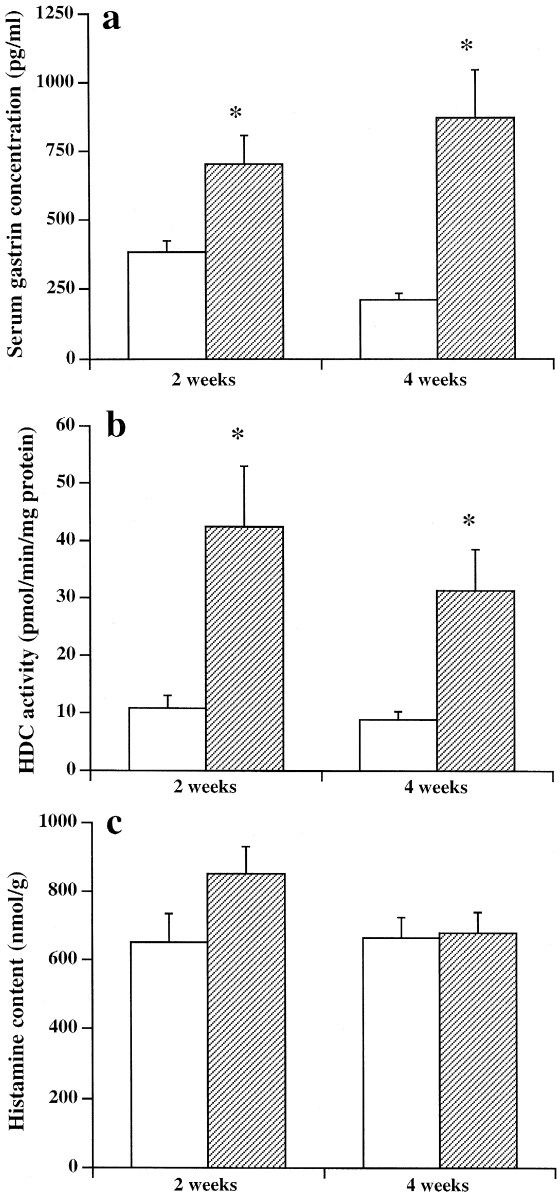

Fig. 1. The serum gastrin concentration as determined by RIA (a), histidine decarboxylase (HDC) activity (b) and histamine content (c) of the oxyntic mucosa in Wistar rats. Animals were treated with rabeprazole (30 mg/kg/day, s.c.) (Z) and the same volume of saline solution (□) for 2 and 4 weeks. Saline-treated rats served as the control group. Values represent means ± SEM ($n = 6$). $^{*}P < 0.05$ compared to the control group.

Two weeks of treatment with rabeprazole caused a 3.9-fold increase in the histidine decarboxylase activity of the oxyntic mucosa over control levels (treated vs. control:

42.26 ± 10.79 pmol/min/mg protein vs. 10.79 ± 2.30) (Fig. 1b). Four weeks of treatment elicited a 3.6-fold increase (31.42 ± 7.03 vs. 8.80 ± 1.25). No significant difference with respect to histidine decarboxylase activity was found between the 2- and 4-week treatment in either treated or control rats.

Neither 2- nor 4-week treatment with rabeprazole affected the histamine content of the oxyntic mucosa (treated for 2 weeks vs. control: 852.13 ± 79.60 nmol/g vs. 653.66 ± 36.46; treated for 4 weeks vs. control: 681.89 ± 59.64 vs. 666.28 ± 26.20), as shown in Fig. 1c.

3.2. Effect of rabeprazole on serum gastrin concentration, oxyntic mucosal histidine decarboxylase activity and histamine content in Ws/Ws and +/+ rats

Fig. 2a shows the effect of rabeprazole on the serum gastrin concentration in the Ws/Ws and +/+ rats. A trend toward an increase above control levels in the serum gastrin concentration after 2 weeks of treatment with rabeprazole was observed in the Ws/Ws rats, although the changes were not statistically significant (treated vs. control: 435.37 ± 68.68 pg/ml vs. 231.04 ± 45.88). However, 4 weeks of treatment induced a greater than 4.4-fold increase (513.09 ± 121.42 vs. 117.01 ± 15.57). Meanwhile, significant increases in the serum gastrin concentration of the +/+ rats were observed, after the 2- and 4-week treatment with rabeprazole, and values were approximately 4.0- and 2.6-fold higher than the control levels, respectively (treated for 2 weeks vs. control: 730.29 ± 140.81 pg/ml vs. 184.81 ± 41.20; treated for 4 weeks vs. control: 681.03 ± 150.99 vs. 261.05 ± 91.37). No significant differences were found among the control groups, which included Ws/Ws and +/+ rats.

Fig. 2b shows that rabeprazole increased histidine decarboxylase activity in the oxyntic mucosa of the Ws/Ws and +/+ rats. Significant increases in oxyntic mucosal histidine decarboxylase activity in the Ws/Ws rats were found after the 2- and 4-week treatment with rabeprazole, and values were 5.1- and 7.2-fold higher than the control levels, respectively (treated for 2 weeks vs. control: 49.01 ± 6.92 pmol/min/mg protein vs. 9.66 ± 2.12; treated for 4 weeks vs. control: 41.18 ± 9.71 vs. 5.73 ± 1.61). Moreover, in the +/+ rats, the histidine decarboxylase activity of the oxyntic mucosa was increased 5.3-fold after the 2-week treatment and 8.4-fold after the 4-week treatment (treated for 2 weeks vs. control: 68.93 ± 6.61 vs. 13.07 ± 3.04; treated for 4 weeks vs. control: 47.90 ± 15.08 vs. 5.68 ± 1.82). There were no significant differences among the control groups, which included Ws/Ws and +/+ rats.

The oxyntic mucosal histamine content was increased 2.2-fold and 2.1-fold in the Ws/Ws rats after the 2- and 4-week treatment with rabeprazole, respectively (treated for 2 weeks vs. control: 498.50 ± 49.62 nmol/g vs. 231.45 ± 28.89; treated for 4 weeks vs. control: 464.70 ± 41.48 vs. 218.24 ± 30.01) (Fig. 2c). Also, 1.6- and 2.2-fold increases were observed in the +/+ rats after the 2- and

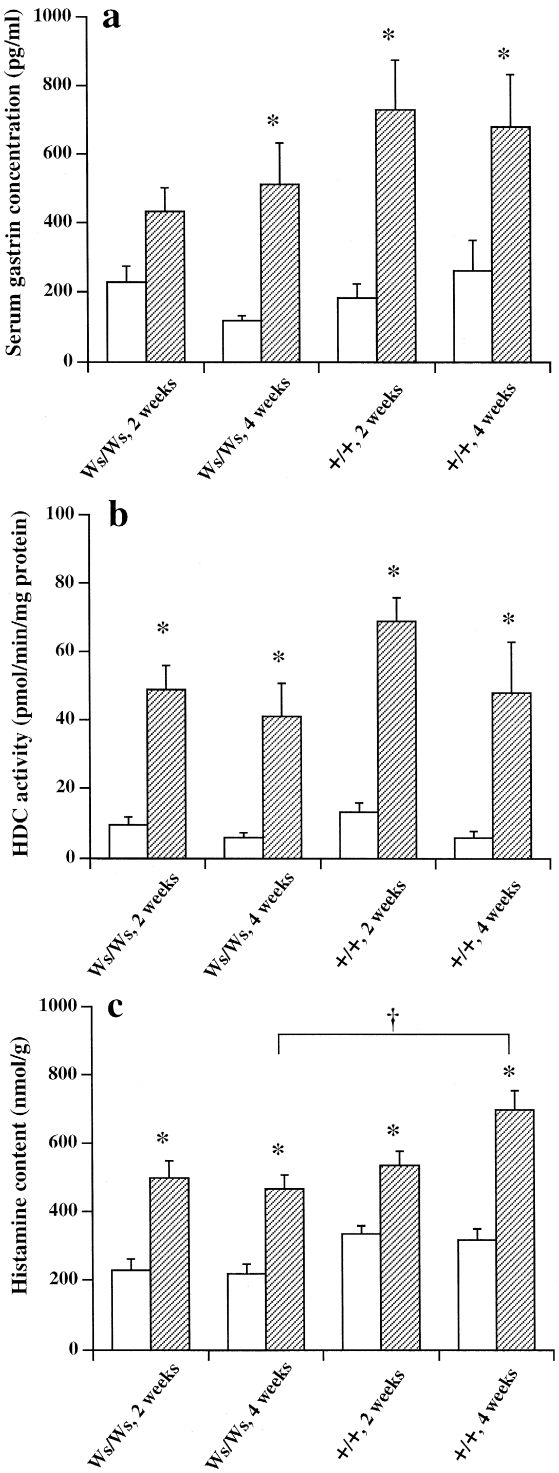

Fig. 2. The serum gastrin concentration as determined by RIA (a), histidine decarboxylase (HDC) activity (b) and histamine content (c) of the oxyntic mucosa in Ws/Ws and +/+ rats. Animals were treated with rabeprazole (30 mg/kg/day, s.c.) (Z) and the same volume of saline solution (□) for two weeks and four weeks. Saline-treated rats served as the control group. Values represent means ± SEM ($n = 6$). * $P < 0.05$ compared to the control group. † $P < 0.05$ comparisons between groups calculated with the Fisher test.

4-week treatment with rabeprazole, respectively (treated for 2 weeks vs. control: 533.01 ± 42.76 vs. 334.33 ± 25.50; treated for 4 weeks vs. control: 695.16 ± 58.57 vs. 318.65 ± 33.22). No significant differences were found among the control groups, which included Ws/Ws and +/+ rats. However, the oxyntic mucosal histamine content for the +/+ rats was higher than that for the Ws/Ws rats in the 4-week treatment study (695.16 ± 58.57 vs. 464.70 ± 41.48).

3.3. Effect of rabeprazole on the density of histamine-containing cells in the oxyntic gland area and oxyntic mucosal thickness in Wistar, +/+, and Ws/Ws rats

Fig. 3 shows histamine immunostaining of the oxyntic gland area. In the control Wistar and +/+ rats, histamine-immunoreactive cells were seen in the superficial layer and basal half of the oxyntic mucosa, and in the submucosa (Fig. 3a,c), whereas in the control Ws/Ws rats, they were seen only in the basal half of the oxyntic mucosa (Fig. 3e). Rabeprazole treatment for 4 weeks induced a marked increase in the density of histamine-immunoreactive cells in the oxyntic mucosa in the Wistar, +/+, and Ws/Ws rats, although there were no changes in the superficial layer of the oxyntic mucosa and submucosa (Fig. 3b,d,f). Table 1 shows the ratio of the histamine-immunofluorescent area to the total oxyntic mucosal area (%Ar) and oxyntic mucosal thickness after the 4-week treatment with rabeprazole in the Wistar, +/+, and Ws/Ws rats. The %Ar of histamine immunofluorescence was 1.2- 1.7- and 1.9-fold higher than the control levels in the Wistar, +/+, and Ws/Ws rats, respec-

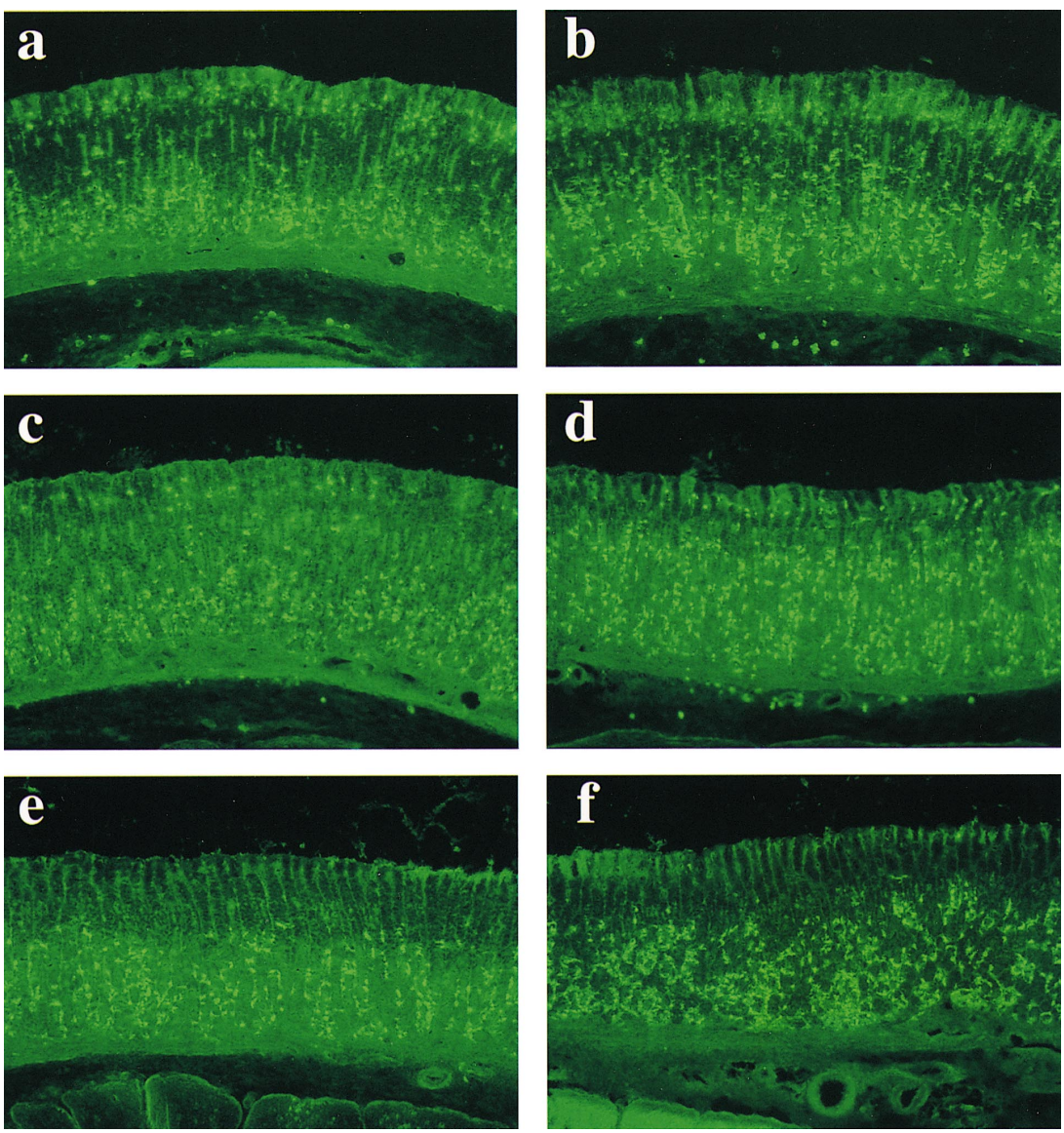

Fig. 3. Histamine immunostaining of the oxyntic gland area in control rats (a, c, e) and rabeprazole-treated (30 mg/kg/day, s.c., for 4 weeks) rats (b, d, f). Saline-treated rats served as the control. (a, b) Wistar rat. (c, d) +/+ rat. (e, f) Ws/Ws rat (magnification ×100).

Table 1
The ratio of the histamine-immunofluorescent area to the total oxyntic mucosal area (%Ar) and oxyntic mucosal thickness after treatment with rabeprazole for 4 weeks in Wistar, +/+, and Ws/Ws rats

	Wistar	+/+	Ws/Ws
(%)Ar			
Control	3.16±0.10	3.09±0.30	2.60±0.18
Rabeprazole	3.82±0.21[a]	5.16±0.11[a]	4.89±0.08[a]
Oxyntic mucosal thickness (μm)			
Control	518.38±13.04	518.10±20.06	505.32±10.14
Rabeprazole	608.19±21.58[a]	568.34±18.78	558.21±27.28

Data represent means±SEM ($n=6$).
[a] $P<0.05$, compared to the value in the respective control group.

tively. The oxyntic mucosal thickness was increased 1.2-fold over the control levels after the 4-week treatment with rabeprazole in the Wistar rats, whereas no significant increase over the control levels in the oxyntic mucosal thickness was found after treatment with rabeprazole for 4 weeks in the +/+ and Ws/Ws rats.

4. Discussion

Gastric acid secretion is regulated by luminal acidity via the synthesis and release of gastrin. Although the mechanism of this regulation remains uncertain, the increase in luminal pH triggers gastrin synthesis and release in G cells and, consequently, stimulates histamine synthesis and release in ECL cells in the stomach. Endogenous hypergastrinemia generated by acid inhibitory treatment induces an increase in histidine decarboxylase activity of the oxyntic mucosa, which is involved in histamine biosynthesis (Håkanson et al., 1994a,b). Furthermore, sustained hypergastrinemia in rats resulted in a 2- to 4-fold increase in the density of histamine-containing ECL cells within 1–2 weeks (Ryberg et al., 1990; Tielemans et al., 1990; Håkanson et al., 1992).

Several irreversible proton pump inhibitors have been developed and clinically used for the treatment of gastric ulcer. Rabeprazole is more potent than omeprazole in inhibiting acid secretion (Fujisaki et al., 1991). The present study first demonstrated the effect of rabeprazole on serum gastrin levels and the function of ECL cells in Wistar rats. The serum gastrin concentration and histidine decarboxylase activity of the oxyntic mucosa were increased significantly after the 2- and 4-week treatment (Fig. 1a,b). This finding is in accordance with that of previous reports with other proton pump inhibitors such as omeprazole and lansoprazole (Larsson et al., 1986; Lee et al., 1992). However, our study showed that there were no significant changes in the histamine content of the oxyntic mucosa after the 2-week or even the 4-week treatment with rabeprazole as compared with control levels (Fig. 1c). As to the reason for this finding, it cannot be excluded that the amount of histamine stored in mast cells of the oxyntic stomach may have masked an increase in the amount of histamine derived from ECL cells. The technique of scraping the mucosa from the inside of a removed stomach is often used when sampling the oxyntic mucosa. This technique presents a problem in that samples always contain mast cells from the superficial layer of the oxyntic mucosa and also could contain some mast cells from the submucosa. There are several reports on the number of ECL cells and mast cells in the oxyntic stomach of rats. Treatment with α-fluoromethylhistidine, which is an irreversible inhibitor of histidine decarboxylase, reduces the histamine content in the oxyntic mucosa by 80% (Andersson et al., 1992a,b, 1996). Andersson et al. also used the method of scraping the oxyntic mucosa and showed by immunocytochemistry, using histamine antiserum, that α-fluoromethylhistidine depletes histamine from ECL cells but not from mast cells. Hence, it is possible to conclude that the remaining 20% of the total amount of histamine, of which the turnover rate is slow, resides in mucosal and submucosal mast cells. However, it has been reported that, in the mast cell-deficient (Ws/Ws) rat, the histamine content in all layers of the oxyntic stomach is approximately half that in the control normal rat (+/+) (Onoue et al., 1993). In other words, the amount of histamine stored in ECL cells is almost equal to that in mast cells in the oxyntic stomach. Thus, it seems that the ratio between ECL cells and mast cells in the oxyntic stomach depends on the method of sampling. In the end, we thought it difficult to estimate the amount of histamine derived from ECL cells in Wistar rats.

In order to solve this problem, Ws/Ws rats were treated with rabeprazole and compared with +/+ rats. Serum gastrin concentration and histidine decarboxylase activity were increased significantly as compared with control levels within 4 weeks after the start of treatment in the Ws/Ws rats, and the extent of the increase was approximately the same as that in +/+ rats (Fig. 2a,b). These results are consistent with data for the Wistar rats. However, a significant increase in the histamine content of the oxyntic mucosa over control levels within 4 weeks after the start of treatment was observed in the Ws/Ws rats and also in the +/+ rats (Fig. 2c). Furthermore, these findings concerning the increase in histamine content of the oxyntic mucosa were confirmed visually by immunohistochemistry using a histamine antibody (Fig. 3). This is the first immunostaining study with histamine antibody performed in Ws/Ws rats and demonstrated that no mast cells exist in the superficial layer of the oxyntic mucosa and submucosa. That is, in the non-treated Ws/Ws rats, histamine-immunoreactive cells were seen only in the basal half of the oxyntic mucosa, whereas in the non-treated +/+ rats, they were seen not only in the basal half layer but also in the superficial layer of the oxyntic mucosa and in the submucosa. In addition, histological examination with toluidine blue staining revealed that mast cells showing metachromasia and orthochromasia were present in the submucosa and in the superficial layer of the oxyntic

mucosa, respectively, of the +/+ rats but not of the Ws/Ws rats (data not shown). According to previous reports on the location of ECL cells and mast cells in the rat stomach (Håkanson et al., 1986a), histamine-immunoreactive cells in Ws/Ws rats are considered to be ECL cells. Morphometric analysis showed that 4-week treatment with rabeprazole resulted in an obvious increase in the quantity of histamine stored in ECL cells over control levels, with almost the same magnitude of increase being seen in the Ws/Ws and +/+ rats.

The results of the present study suggest that ECL cells in Ws/Ws rats maintain their response to gastrin in the intermediate and chronic phases of the response of the gastrin–ECL cell axis (Håkanson et al., 1992, 1994a,b; Chen et al., 1994) in vivo in spite of the lack of mast cells. Their response to gastrin in the acute phase in vivo has been investigated by using the in vivo microdialysis method (Watanabe et al., 1996) in our laboratory. For the present, we have very recently found that an infusion of pentagastrin (62.5 μg/kg/h) into the jugular vein of Ws/Ws rats increased the concentration of plasma histamine and gastric acid output (data not shown).

Moreover, the finding that there were no differences in serum gastrin concentration and in histidine decarboxylase activity between the Ws/Ws and +/+ rats, both with and without the 4-week treatment, indicates that mast cells do not respond to endogenous hypergastrinemia elicited by acid-inhibitory treatment. Also, in vitro it has been reported that pentagastrin does not stimulate histamine release from peritoneal mast cells of rats (Watanabe et al., 1996) and mucosal mast cells prepared from the canine oxyntic mucosa do not show a response to gastrin (Soll et al., 1988).

Gastrin stimulates the proliferation of mucosal cells and increases oxyntic mucosal thickness (Larsson et al., 1986). As shown in Table 1, the increase in oxyntic mucosal thickness elicited by rabeprazole was more pronounced in the Wistar rats (1.17-fold) than in the Ws/Ws (1.10-fold) and +/+ (1.11-fold) rats. The high proliferation rate in the Wistar rats resulted in a lower value for the ratio of histamine-immunofluorescent area to the total oxyntic mucosal area than that for the Ws/Ws and +/+ rats, although the reason why the gastric mucosal thickness in response to hypergastrinemia differs in these strains remains uncertain. In addition, this difference in gastric mucosal thickness is the reason why no difference in the histamine content of the oxyntic mucosa between treatment and non-treatment with rabeprazole was seen in the Wistar rats, although a significant increase was seen in the +/+ rats.

As mentioned previously, mast cells are a major source of histamine in the stomach, but the precise role of the histamine in the regulation of oxyntic mucosal function has not been defined. Mast cells play an important role not only in triggering allergic reactions but also in the full development of certain non-allergic inflammatory responses by secreting chemical mediators such as histamine, leukotrienes, and cytokines (Serafin and Austen, 1987). Connective tissue-type mast cells and mucosal-type mast cells have apparently different biochemical characteristics and responses to secretagogues (Enerbäck, 1981). Connective tissue-type mast cells store heparin and relatively large amounts of histamine in granules and exhibit dependence on stem cell factor for differentiation. By contrast, mucosal-type mast cells contain chondroitin sulfate instead of heparin, and relatively small quantities of histamine, and exhibit dependence on T cell-derived cytokines such as interleukin-3 and interleukin-4 for inductive but not constitutive differentiation (Kitamura et al., 1987; Metcalfe et al., 1997). It has been reported that a significant number of mucosal-type mast cells develop in the jejunum of the Ws/Ws rat during T cell-dependent immune responses to *Nippostrongylus brasiliensis*, although the number is significantly lower than that in the jejunum of *N. brasiliensis*-infected +/+ rats (Arizono et al., 1993). In the light of these findings, it can be speculated that mucosal-type mast cells play a role at mucosal surfaces as a defender against parasitic infections and presumably also food proteins which activate the helper T cell 2 system. Moreover, Ws/Ws rats could be useful for studying the contribution of mucosal-type mast cells to gastric mucosal damage induced by *Helicobacter pylori*.

In conclusion, the present study showed that ECL cells in the rat stomach respond to hypergastrinemia in vivo. Histamine derived from mast cells is unlikely to be involved in gastrin-stimulated histamine synthesis under normal conditions. Finally, mast cell-deficient (Ws/Ws) rats could be used in studies to determine the role of ECL cells in acid secretion. In addition, it should be possible to investigate the contribution of histamine derived from mast cells to acid secretion, i.e., whether the mast cell–parietal cell axis exists under inflammatory conditions.

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